Har Gobind Khorana 1922–2011

Gobind Khorana was a scientist who traversed boundaries, both scientifically and culturally. He pioneered the use of concepts and tools from chemistry and physics to tackle fundamental questions of biology. In particular, he helped to decipher how RNA encodes for the synthesis of protein, research that earned him the Nobel Prize in Medicine with Marshall Nirenberg and Robert Holley in 1968. Although deeply modest and unassuming, Gobind would often remark, "I only work on big problems." Indeed, only such challenges were worthy of the extraordinary intensity, creativity, and focus that Gobind brought to a question.

Gobind is an international icon for science sans frontiers. He was an early practitioner of what is now known as Chemical Biology. He pioneered new synthetic routes for nucleotide cofactors and oligonucleotides and then used these synthetic molecules to help elucidate the genetic code. He assembled the first synthetic gene, thereby developing what might be described as the first "biobrick" and laying the foundation for Synthetic Genomes and Synthetic Biology. In 1971, shortly after his departure from University of Wisconsin-Madison (UW-Madison) to the Massachusetts Institute of Technology (MIT), he described the amplification of synthetic genes in a series of steps that were redefined 15 years later, with a thermostable DNA polymerase, as PCR. At MIT, Gobind took on lipids and membrane proteins, focusing on the structure and function of bacteriorhodopsin and then mammalian rhodopsin until his retirement in 2007.

The intellectual elegance of Gobind's work has been an inspiration for many generations of chemists and biologists, but the high visibility of his success has had a much broader and deeper impact on India, especially while it was struggling to gain its footing after India's independence from the United Kingdom. In India, Gobind is a symbolic figure for how an education can help to overcome socioeconomic and intellectual boundaries. His success was hard won and quite improbable.

Gobind began his education under a tree in a small village of a hundred families, who were mostly illiterate. Fortunately, his talent was recognized early, and he went on to Punjab University in Lahore and almost became an English major because he was too shy to interview for the Chemistry program. Nevertheless, the selection committee overlooked this because of his talent, and he graduated with a Masters in Chemistry. He then won a rare fellowship to pursue a Ph.D. in organic chemistry at the University of Liverpool in England.

After obtaining his doctorate in 1948, Gobind enthusiastically moved to Eidgenössische Technische Hochschule (ETH) in Zurich to join the group of Vladimir Prelog, a chemist who won a Nobel Prize in 1975 for his work on stereochemistry. In less than a year, he had to leave Zurich because his savings ran out. During his brief tenure at the ETH, however, Gobind serendipitously encountered the little-known work of Fritz Zetzsche on carbodiimides. This class of compounds became incredibly important for Gobind to synthesize nucleotide cofactors (such as coenzyme A) and ATP with "astonishing rapidity," as he would later state in his reflections.

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Gobind was unable to find a position in India after training at ETH, a setback that became a blessing in disguise. He instead received a three-year fellowship with Alexander Todd at Cambridge University. While at Cambridge, Gobind was exposed to Sanger's exciting advances in protein sequencing, Perutz's and Kendrew's breakthroughs in protein crystallography, and Todd's own work on the chemical structures of nucleic acids. This innovative environment drew Gobind, a synthetic organic chemist, to the newborn field of Molecular Biology.

In 1952, Gobind began his independent scientific career as a nonacademic researcher at the British Columbia Research Council in Vancouver, Canada. His meteoric rise and sustained string of seminal scientific contributions might be grouped roughly into two phases. The first phase focused on nucleotides and nucleic acids. He used a carbodiimide (i.e., dicyclohexylcarbodiimide) to form pyrophosphate bonds, which eventually led to the first synthesis of coenzyme A and ATP. Soon, Gobind wrote to Van Potter, a leading cancer biologist at UW-Madison, asking if he would test his synthetic ATP in rigorous biochemical assays. Van Potter not only obliged with the experiments, but also succeeded in bringing Gobind to the Institute for Enzyme Research, a vanguard of chemical biology at UW-Madison.

From 1960 to 1970, Gobind was co-Director of the "Enzyme Institute" and a member of the Department of Biochemistry at UW-Madison. During this period, he generated synthetic oligonucleotides and amplified these molecules biosynthetically with DNA polymerase, which he learned to purify in Arthur

Gobind with two Khorana scholars

Kornberg's lab. Using the oligonucleotide CUCUCU, he discovered that the triplets CUC and UCU encode the amino acids leucine and a serine, respectively. This work corroborated the studies of Marshall Nirenberg, who had previously shown that a UUU triplet encoded a phenylalanine residue. Gobind, with his characteristic humility, would always note that Nirenberg's work inspired his own investigations of the genetic code.

By this time, Gobind was already setting his sights on synthesizing a complete gene, even though the understanding of a gene was still far from concrete. Using DNA ligases, Gobind's lab assembled the coding region of the gene for the alanine tRNA. By 1976, Gobind had added the required regulatory elements needed to express the gene in a living bacterial cell and demonstrated that the synthetic tRNA functioned identically to the naturally expressed gene. This tour de force work defined the conceptual and technical framework for biotechnology and, 40 years later, is still the strategy used to assemble synthetic genes and genomes.

The second phase of Gobind's career began at MIT. He focused on two general areas: to identify the mechanism by which intrinsic membrane proteins function and to map their interactions on a molecular scale with phospholipids of the lipid bilayer structure. Gobind became particularly intrigued by light-sensitive bacteria, with a specific interest in the purple membranes of *Halobacterium halobium*. This project led to the characterization of *H. halobium* bacteriorhodopsin and, eventually, mammalian rhodopsin—the last great problem tackled by the Khorana lab.

The strategy that he chose for this project was a forerunner of contemporary systems biology: sequence the protein, mutate every residue, express and reconstitute the proteins in a native biological context, and then meticulously monitor the phenotypic perturbations, with the eventual goal to provide a comprehensive solution to the problem of bioenergetics. In the process, he corrected misimpressions related to the mechanism of rhodopsin, including the location of the retinal chromophore in bacteriorhodopsin and the transmembrane structure of bacteriorhodopsin. Through the efforts of countless colleagues and collaborators, Gobind elucidated the detailed mechanism by which bacteriorhodopsin pumps protons across the membrane when activated by light. Similar approaches were then used to determine the mechanism and structure-function relationships in mammalian rhodopsin, a problem that engaged Gobind until his death. Characteristically, Gobind tackled this question with the fervor and energy of a newly minted assistant professor.

Inspired by Gobind's story, UW-Madison launched the *Khorana Scholars Program* in 2007, the year Gobind retired from active research. Gobind generously lent his name to the program, which aims to identify and nurture future leaders in both the United States and India. This exchange program identifies talented American and Indian students and places them in leading laboratories for a transformative summer research experience in the host country. The Khorana program is now expanding to partner with top US universities and leading Indian scientific institutes. The program also sends Wisconsin agricultural scientists to India to assist in entrepreneurial efforts aimed at improving economic stability and food security in poor rural areas (http://www.biochem.wisc.edu/faculty/ansari/khorana_program). During his last visit to UW-Madison, Gobind met the 2009 scholars. His joy at meeting the future generation of scientists stemmed from seeing their passion for science and perhaps recognizing a bit of himself in them.

Gobind's passion for science and the exacting standards that he set enabled many of his students to become leaders in industry and academia. His postdoctoral fellow Michael Smith won the Nobel Prize in 1993 for site-directed mutagenesis, ironically sharing the award with Kary Mullis, who was honored for inventing PCR. Other trainees founded major biotech companies, such as Amgen, and became presidents of leading educational institutions and research centers.

Gobind's mentorship involved rigorous intellectual training and hard work. It did not matter if none of the experiments worked (at least for the impossible problems!), but complete "24/7" engagement was expected. One possibly apocryphal story involved Saturday morning donuts that Gobind would bring to the lab. Rumor had it that he had identified everyone's favorite type of donut and would bring only one each. At the end of the day, he would check the remaining donuts and determine who had come in over the weekend!

Although the lab worked hard, it also shared many memorable Friday night sessions in a room above the Muddy Charles pub at MIT—meetings where spouses, children, and dogs were all welcome.

Gobind's lab drew from many disciplines and countries, and thus it populated faculty positions across the world. He treated women exactly the same as men ("Want to race up the stairs?" he often asked M.R.R.); a former postdoctoral fellow even became one of Japan's first female biochemistry professors.

The respect and warmth felt by Gobind's academic family was readily evident in the periodic "Khorana Symposia," which were held in various parts of the world. The last such gathering was in 2009 at the 33rd Steenbock symposium on *Synthetic Gene to Synthetic Genomes*. Many of the scientists that spoke, in describing their current work, noted how Gobind shaped their thinking and how his contributions continue to propel new fields, such as synthetic and chemical biology. As a tribute to Gobind, UW-Madison has made these talks publicly accessible at http://www.biochem.wisc.edu/seminars/steenbock/symposium33.

Gobind had a deep interest in nature and its beauty; he actively sought solitude in natural settings to think deeply and critically about science. His beloved wife Esther, whom he lost in 2001, ensured that he was free to focus exclusively on science. He would often rent a room or cottage without a phone, radio, or TV so that he could think and write without distraction. In fact, Esther had to drive an hour to inform Gobind that he had won the Nobel Prize. Gobind tragically lost his daughter Emily in 1978 but is survived by his loving children David and Julia, who cared for him in his final years.

Har Gobind Khorana was an influential scientist, a rigorous educator, and a humanitarian. He pursued his work with a single-minded intensity but remained humble to the end. His innate curiosity and appreciation of life (as well as that quick laugh, that slight tilt as he craned his head to listen, and that quizzical look) will remain with those who were fortunate to be his friend. His legacy will live on through generations. If one wants an appreciation of the great scientific oeuvre of Gobind Khorana, the best place to look is a book of selected papers complete with introductions compiled by Gobind a few years ago (Khorana, H.G. [2000]. Chemical Biology: Selected Papers of H. Gobind Khorana [with Introductions] [Singapore: World Scientific Publishing Co. Pte. Ltd.]).

At the beginning of the Preface is a telling quote from Otto Loewi: "We must be modest except in our aims."

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